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Original Research Paper

Development and *in-vitro* evaluation of an optimized carvedilol transdermal therapeutic system using experimental design approach

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ABSTRACT

The effect of formulation variables on *in-vitro* release and permeation properties of carvedilol from transdermal patch was studied by varying one factor at a time as preliminary study. Based on these results, design of experiments technique was applied followed by regression analysis and response surface methodology to optimize formulation variables. Central Composite IV model design was used with four formulation variables: drug loading, matrix thickness, adhesive layer thickness, and propylene glycol concentration. Nineteen formulations were prepared according to the design; and the effect of formulation variables was studied on *in-vitro* release and permeation profiles of these formulations. In all cases, the permeation profiles paralleled *in-vitro* release profiles. The drug released at 7 h and 24 h was used as release response parameters while permeation flux obtained was employed as permeation response parameter. All four formulation variables were found to be significant for release properties and three of these exhibited significant effect on permeation profile of carvedilol across artificial membrane. Constrained optimization, using 47.9% of cumulative carvedilol released at 7 h and 99.8% at 24 h as well as 25.7 $\mu g/cm^2/h$ of permeation flux, was applied to obtain desired release and permeation profiles. Experimentally, carvedilol was observed to release from the optimized formulation with 51.4% drug release at 7 h and 98.5% at 24 h with an observed flux value of 27.4 $\mu g/cm^2/h$ across artificial membrane, which showed an excellent agreement with the predicted values. The results of this investigation show that the quadratic mathematical model developed could be used to further predict formulations with desirable release and permeation properties.

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1. Introduction

Carvedilol is a potent beta-adrenergic blocking agent commonly used in hypertension, left-ventricular dysfunction, and several other cardio-vascular disorders. Currently, carvedilol is administered orally in the form of tablets. The recommended dose for carvedilol is 3.125–6.25 mg twice a day for 7–14 days for hypertension, left-ventricular dysfunction or following myocardial infarction. Although carvedilol is completely absorbed from the gastrointestinal tract after ingestion of traditional peroral tablets, the systemic availability is only about 25%. This leads to several dose-related side effects, such as bradycardia, cardiac insufficiency, cardiogenic shock and cardiac arrest. Several attempts have been made to resolve the above mentioned disadvantages, including development of monolithic matrices of carvedilol by supercritical fluid, carvedilol–cyclodextrin complex, and buccal sprays of oral suspension [1–3].

The biological properties of carvedilol, such as high first-pass metabolism and low therapeutic dose, and its suitability for patients requiring long-term treatment and repetitive dosing, make carvedilol an interesting candidate for transdermal administration. Furthermore, the high lipophilicity (log $P = 3.97$) and low molecular weight (MW 406.5) also indicate a good probability of carvedilol crossing the lipophilic skin barrier [4]. Therefore, it should be possible to control release of carvedilol over a long period of time thereby decreasing frequency of administration and improving patient compliance which could prove beneficial to the patient.

Thus, the objective of this investigation was to develop a transdermal drug delivery system to deliver carvedilol at a controlled rate as well as to evaluate formulation variables which affect *in-vitro* release and permeation profiles of carvedilol. A matrix type design was selected for this investigation due to its ease of manufacturing and high tensile strength. Also, it has been reported that a high release or flux of a lipophilic drug could be obtained if the drug is loaded in a hydrophilic matrix [5]. Therefore, hydroxypropyl methylcellulose (HPMC) was selected as the matrix polymer. Propylene glycol was used as the permeation enhancer and a commonly used polyacrylate was employed as the adhesive.

As a preliminary study, the influence of four formulation factors (drug loading, matrix thickness, adhesive layer thickness, and propylene glycol concentration) was investigated on the release and permeation properties of carvedilol from transdermal patches by changing one factor at a time. After completion of the preliminary study, an attempt was made to obtain an optimized formulation by design of experiments so as to achieve a desired release (50% in 7 h and 100% in 24 h) and permeation flux (25.7 $\mu g/cm^2/h$) of carvedilol from the patch over a particular period of application time of the patch. The advantages of using this experimental design method in contrast to the one-factor-at-a-time classic experimental approach include the following: reduction in the number of experiments that need to be carried out, identification of interaction between formulation factors, detection of optimal response within the experimental region, and empirical modeling of the data.

2. Materials and methods

2.1. Materials

Carvedilol was obtained as free sample from Caraco Pharmaceuticals (Detroit, MI, USA). Polyester backing membrane (3M Scotchpak™ 9733 backing) and release liner (3M Scotchpak™ 1020 release liner) were obtained as free samples from 3M (St. Paul, MN, USA). Propylene glycol, hydroxypropyl methylcellulose (HPMC) and phosphate buffer solution (pH 6.8) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The solvents and agents used for the determination of carvedilol content in the samples by HPLC method were HPLC grade and obtained from VWR International (West Chester, PA, USA). The adhesive (Duro-tak® 87-2516) used for this study was obtained as a free sample from The National Starch and Chemical Company (Bridgewater, NJ, USA). The transdermal patch retainer used for the *in-vitro* release study was purchased from Quality Lab Accessories (Bridgewater, NJ, USA). The artificial membrane (MF-Millipore™ membrane filter, filter code VSWP) used for the *in-vitro* permeation study was purchased from Millipore Corporation (Bedford, MA, USA).

2.2. Analysis of carvedilol

The concentration of carvedilol in the samples was analyzed using HPLC method (HP1100 series, Agilent Technologies, Wilmington, DE) with a Symmetry C_{18} column (5.0 μm, 4.6 × 250 mm). The mobile phase consisted of methanol, 0.33 N phosphate buffer (4.5 g KH_2PO_4 and 0.61 g K_2HPO_4 dissolved in 1000 mL purified water), and glacial acetic acid at a ratio of 60:40:0.3 (by volume) and the flow rate was 1 mL/min [6]. Carvedilol was detected at 284 nm with a retention time of 4.7 min. The volume of drug solution injected was 10 μL for both, standards and samples. The concentration of carvedilol was quantified by the peak area method from the associated calibration curve.

2.3. Fabrication of carvedilol-loaded transdermal systems

Solvent casting method was used to prepare transdermal systems. Briefly, carvedilol was added as an alcoholic solution to facilitate incorporation of carvedilol into the 2% w/v aqueous solution of HPMC. Propylene glycol was then added and the solution was mixed well. Films of required thicknesses were cast on a polyester backing membrane using a bar film applicator (Byk Gardner, Columbia, MD, USA) of required clearance (in unit of micrometers). For instance, a 400 μm of HPMC matrix thickness was obtained by using a bar film applicator for 400 μm clearance and so on for other thickness values. Adhesive layer of required thicknesses was then applied, after drying the films in an oven at 40 °C for 2 h to evaporate any solvents used, using the same method for thickness of HPMC matrix. Films of desired size were sectioned using sharp blade and release liner was then

applied to prevent loss of drug from the film. The carvedilol-loaded transdermal systems were then stored in a desiccator until further use.

2.4. Preliminary study

Transdermal patches having 800 μm matrix thickness, 25 μm adhesive layer thickness, and 2.5% propylene glycol concentration were prepared with varying carvedilol drug loading concentrations at 8%, 10%, 12.5% and 15% levels to study the effect of carvedilol loading in the HPMC-matrix on the release and permeation profiles of carvedilol. The effect of matrix thickness (400 μm, 800 μm, and 1600 μm) was studied on carvedilol release as well as permeation from the patches in which carvedilol loading, adhesive layer thickness, and propylene glycol concentration were kept constant at 12.5% of the polymer, 25 μm, and 2.5%, respectively. Adhesive layer having a thickness of 12.5 μm, 25 μm, 50 μm, or 100 μm was applied on different matrices and carvedilol release as well as permeation was studied while carvedilol loading, matrix thickness, and propylene glycol concentration were kept constant at 12.5% of the polymer, 800 μm, and 2.5%, respectively. The effect of various propylene glycol concentrations (1.5%, 2.5%, 3.5%, and 4.5%) was studied on carvedilol release and permeation from the patches keeping the other values of carvedilol loading, matrix thickness, and adhesive layer thickness constant at 12.5% of the polymer, 800 μm, and 25 μm, respectively. The composition of formulations used in the preliminary study is displayed in Table 1.

2.5. Statistical optimization of the formulation variables using experimental design approach

Following the preliminary study, further evaluation of the four formulation variables was performed using the principle of design of experiments to identify an optimal combination of formulation variables for the fabrication of patches having desired drug release rate and permeation flux. A Central Composite IV model of Fusion Pro™ Software (S-matrix Corporation, Eureka, CA, USA) was selected which consisted of 8 full factorial design points, 8 axial points and 3 center points (Table 2). This design involved three dependent variables (Y_1, Y_2, and Y_3) and four independent variables (X_1, X_2, X_3, and X_4). The response surface can be expressed as $Y = f(X_1, X_2, X_3, X_4)$. The four independent variables selected for this study were X_1, carvedilol loading; X_2, matrix thickness; X_3, adhesive layer thickness; and X_4, propylene glycol concentration in the patches. All other formulation and processing parameters were kept invariant throughout the study. The three dependent variables included the following: Y_1, carvedilol released at 7 h; Y_2, carvedilol released at 24 h; and Y_3, permeation flux of carvedilol across artificial membrane. The composition of 19 patch formulations based on this model is displayed in Table 3 which was used for the fabrication of carvedilol-loaded transdermal systems. Upon the completion of statistical optimization experiments, regression equations and 3-dimensional response surface plots were generated to study the contributions of these variables to different response parameters in order to identify the optimized carvedilol-loaded transdermal system. The optimized system thus

Table 1 – Composition of formulations generated from preliminary studies and results of response parameters obtained from *in-vitro* release as well as permeation profiles of respective formulation of transdermal systems.

Formulation code	Formation variable				Response parameter		
	Carvedilol loading (% w/w of polymer)	HPMC matrix thickness (μm)	Adhesive layer thickness (μm)	Propylene glycol concentration (% v/v of solution)	Cumulative carvedilol released at 7 h (% ± S.D.)	Cumulative carvedilol released at 24 h (% ± S.D.)	Permeation flux (μg/cm^2/h ± S.D.)
Effect of carvedilol loading							
P1	8	800	25	2.5	21.9 ± 0.8	32.7 ± 2.4	4.2 ± 0.3
P2	10	800	25	2.5	32.5 ± 4.3	52.8 ± 5.9	11.0 ± 1.0
P3	12.5	800	25	2.5	45.0 ± 2.9	92.4 ± 5.3	23.5 ± 1.5
P4	15	800	25	2.5	53.4 ± 3.2	100.7 ± 7.3	27.2 ± 2.9
Effect of HPMC matrix thickness							
P5	12.5	400	0	2.5	95.0 ± 1.1	100.1 ± 1.9	115.5 ± 9.3
P6	12.5	800	0	2.5	90.7 ± 2.7	98.8 ± 3.0	68.9 ± 5.2
P7	12.5	1600	0	2.5	84.3 ± 3.5	96.7 ± 2.7	45.1 ± 5.0
Effect of adhesive layer thickness							
P8	12.5	800	0	2.5	90.7 ± 2.7	98.8 ± 3.0	68.9 ± 5.2
P9	12.5	800	12.5	2.5	79.8 ± 3.1	96.8 ± 4.4	53.9 ± 4.2
P10	12.5	800	25	2.5	43.8 ± 2.9	92.2 ± 3.8	21.0 ± 3.2
P11	12.5	800	50	2.5	19.8 ± 0.9	27.9 ± 1.1	4.4 ± 0.2
P12	12.5	800	100	2.5	17.1 ± 1.0	22.1 ± 1.7	1.8 ± 0.1
Effect propylene glycol concentration							
P13	12.5	800	25	1.5	27.9 ± 2.0	42.6 ± 3.5	8.8 ± 3.2
P14	12.5	800	25	2.5	39.9 ± 1.8	76.4 ± 2.9	17.4 ± 0.9
P15	12.5	800	25	3.5	48.0 ± 0.8	97.0 ± 5.6	25.8 ± 1.5
P16	12.5	800	25	4.5	64.5 ± 3.0	102.5 ± 6.3	41.7 ± 2.8

Table 2 – A Central Composite IV model.

Formulation code	X_1	X_2	X_3	X_4
Factorial points				
F1	−1	−1	−1	−1
F2	−1	−1	+1	+1
F3	−1	+1	−1	+1
F4	−1	+1	+1	−1
F5	+1	−1	−1	+1
F6	+1	−1	+1	−1
F7	+1	+1	−1	−1
F8	+1	+1	+1	+1
Axial points				
F9	−2	0	0	0
F10	+2	0	0	0
F11	0	−2	0	0
F12	0	+2	0	0
F13	0	0	−2	0
F14	0	0	+2	0
F15	0	0	0	−2
F16	0	0	0	+2
Center points (replicates)				
F17	0	0	0	0
F18	0	0	0	0
F19	0	0	0	0

identified was fabricated and subjected to validation of statistical optimization design.

2.6. In-vitro *drug release studies*

In-vitro drug release studies from carvedilol-loaded transdermal systems were conducted using USP Apparatus 5, paddle over disk method, (Distek Evolution 6100 Dissolution System, North Brunswick, NJ, USA). The transdermal patch retainer used consisted of 25 cm^2 patch placed between a 17-mesh screen and a glass evaporating dish, clipped together using plastic clips. The dissolution medium (600 mL) was phosphate buffer solution (pH 6.8) maintained at a temperature of 32 ± 0.5 °C (corresponding to skin temperature) and the paddle speed was 50 rpm. Aliquots (1 mL each) were collected at predetermined interval for 24 h and assayed for carvedilol concentration using HPLC method described above. Each release study was performed in triplicate.

2.7. In-vitro *permeation studies*

The main purpose of *in-vitro* permeation studies was to further screen the formulations and to correlate permeation pattern of carvedilol from the carvedilol-loaded transdermal systems with the observed release patterns. It has been reported that carvedilol diffuses transdermally mainly by passive diffusion [4]. An artificial membrane was used instead of animal skin or human skin in this investigation since the main purpose was initial screening of the formulations. Also, it has been reported that the permeation profile through a mixed cellulose acetate-cellulose nitrate artificial membrane (MF-Millipore™ membrane filter, filter code VSWP) could be correlated with permeation through human skin [7] Hence, this membrane was selected for the *in-vitro* permeation study in this investigation. The membrane was hydrated overnight in phosphate buffer solution and then placed over the receptor compartment of Franz diffusion cell with a diffusion area of 0.64 cm^2 and a receptor compartment capacity of 11.5 mL. The carvedilol-loaded transdermal system was placed over the membrane and sealed with parafilm. The medium used in the receptor compartment was phosphate buffer solution, which was maintained at 32 ± 0.5 °C by circulating water jackets. Samples (0.5 mL each) were withdrawn from the receptor compartment at predetermined intervals and replaced with an equal volume of fresh phosphate buffer solution to maintain sink conditions. The carvedilol content of the withdrawn samples was determined by HPLC method. Each experiment was performed in triplicate. The cumulative amount of carvedilol permeated per unit area from the transdermal system through the artificial membrane into the receptor compartment medium was plotted as a function of time, and the slope of the linear portion of the plot was estimated as steady state flux.

2.8. *Regression analysis of the optimization of formulation*

The contribution of different formulation variables was compared using analysis of variance (ANOVA) and the level of significance was taken as $P < 0.05$. Regression analysis was carried out to obtain a quadratic model in the form shown in Equation (1):

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5E + b_6\text{Curvature} \quad (1)$$

In Equation (1), Y is the measure of response associated with each factorial level combination; b_0 is an intercept; b_i is the regression coefficient computed from observed experimental values of Y; X_1, X_2, X_3, and X_4 stand for main effects of the formulation variables; E stands for interaction between the formulation variables like X_1X_3 and X_2X_4 etc; Curvature is the quadratic term of the independent variables like $(X_1)^2$ and $(X_2)^2$ etc, which was used to simulate the curvature of the designed sample space. In addition to regression analysis, a backward elimination procedure was used to fit the obtained data to the quadratic model.

3. Results and discussion

3.1. *Preliminary study*

The preliminary study was conducted to evaluate the effect of various formulation factors such as carvedilol loading, matrix thickness, adhesive layer thickness, and propylene glycol concentration on the release of carvedilol from the patches as well as their permeation profile across an artificial membrane. This study was conducted to evaluate one formulation variable at a time. Thus, only one parameter was varied keeping other parameters constant.

As outlined in Table 1, a total of 16 formulations (formulations P1–P16) were investigated as the preliminary study. The effect of four formulation variables studied on drug release as well as permeation profile of carvedilol from carvedilol-loaded transdermal systems is shown in Figs. 1 and 2. Furthermore, the carvedilol released at 7 h and 24 h as well

Table 3 – Composition of formulations generated based on Central Composite IV model and results of response parameters obtained from *in-vitro* release as well as permeation profiles of respective formulation of transdermal systems.

Formulation code	Formation variable				Response parameter		
	Carvedilol loading (% w/w of polymer)	HPMC matrix thickness (μm)	Adhesive layer thickness (μm)	Propylene glycol concentration (% v/v of solution)	Cumulative carvedilol released at 7 h (% ± S.D.)	Cumulative carvedilol released at 24 h (% ± S.D.)	Permeation flux (μg/cm^2/h ± S.D.)
Factorial points							
F1	10	500	12.5	2	63.3 ± 4.4	96.6 ± 7.4	50.7 ± 3.2
F2	10	500	37.5	5	23.2 ± 1.9	38.2 ± 2.8	10.6 ± 0.9
F3	10	1100	12.5	5	78.8 ± 5.3	99.2 ± 8.9	70.3 ± 6.2
F4	10	1100	37.5	2	21.0 ± 1.0	29.6 ± 1.3	5.2 ± 0.2
F5	15	500	12.5	5	89.6 ± 6.4	97.6 ± 9.9	78.6 ± 5.7
F6	15	500	37.5	2	22.3 ± 1.4	33.0 ± 3.0	6.6 ± 0.3
F7	15	1100	12.5	2	69.6 ± 4.4	96.4 ± 6.8	50.9 ± 4.1
F8	15	1100	37.5	5	25.1 ± 1.5	45.2 ± 2.8	11.1 ± 0.9
Axial points							
F9	7.5	800	25	3.5	36.8 ± 1.4	69.1 ± 3.9	13.6 ± 1.0
F10	17.5	800	25	3.5	59.5 ± 3.6	99.8 ± 8.8	35.6 ± 2.3
F11	12.5	200	25	3.5	56.2 ± 2.5	98.0 ± 7.8	24.0 ± 2.5
F12	12.5	1400	25	3.5	39.7 ± 1.4	78.3 ± 5.3	20.1 ± 1.5
F13	12.5	800	0	3.5	93.4 ± 7.3	98.9 ± 7.3	79.4 ± 5.7
F14	12.5	800	50	3.5	18.9 ± 0.9	25.5 ± 2.5	3.5 ± 1.4
F15	12.5	800	25	0.5	27.3 ± 1.4	50.3 ± 3.5	11.1 ± 1.0
F16	12.5	800	25	6.4	73.4 ± 4.3	100.6 ± 8.4	61.4 ± 2.7
Center points (replicates)							
F17	12.5	800	25	3.5	47.6 ± 3.2	96.1 ± 5.0	25.6 ± 1.4
F18	12.5	800	25	3.5	51.9 ± 2.5	96.4 ± 4.1	26.3 ± 1.9
F19	12.5	800	25	3.5	51.3 ± 3.0	98.4 ± 3.2	24.7 ± 1.7

as permeation flux, selected as dependent variables, are also summarized in Table 1.

3.1.1. *Effect of carvedilol loading*

The effect of carvedilol loading on release of carvedilol from patches is represented in Fig. 1A, which shows that the release of carvedilol increased with increase in carvedilol loading. The carvedilol released at 7 h increased from 21.9 ± 0.8% (formulation P1) to 53.4 ± 3.2% (formulation P4). Similar results were seen in carvedilol released at 24 h. Carvedilol release increased from 32.7 ± 2.4% (formulation P1) to 100.7 ± 7.3% (formulation P4). These results may be attributed to the change in carvedilol–polymer ratio with a change in carvedilol loading. As the carvedilol loading is decreased, the polymer fraction in the carvedilol–polymer ratio increases. It stands to reason that higher percentage of polymer produces a dense polymeric network upon hydration with water, which will result in reduced diffusivity of carvedilol [8].

The permeation profiles of carvedilol across the artificial membrane, resulting from the effect of carvedilol loading are shown in Fig. 2A. The results obtained are in accordance with those observed in the release study. The permeation flux of carvedilol increased from 4.2 ± 0.3 μg/cm^2/h (formulation P1) to 11.0 ± 1.0 μg/cm^2/h (formulation P2), 23.5 ± 1.5 μg/cm^2/h (formulation P3), and 27.2 ± 2.9 μg/cm^2/h (formulation P4), when carvedilol loading increased from 8% to 10%, 12.5%, and 15%, respectively. Thus, an increase in carvedilol loading in the patches led to an increase in permeation flux. Similar results were reported in a study where high skin permeation of benztropine was obtained with a higher drug loading in the patch formulations [9]. According to Fick's law of diffusion, the permeation of the drug is directly proportional to the drug concentration gradient across the membrane. Since sink condition was maintained in this study, the concentration of the drug on the donor side of the membrane determined the rate at which the drug diffused through the membrane.

3.1.2. *Effect of HPMC matrix thickness*

The effect of matrix thickness on release of carvedilol from the patches is displayed in Fig. 1B. The rate of release decreased with an increase in the matrix thickness. The carvedilol released at 7 h decreased from 95.0 ± 1.1% (formulation P5, matrix thickness = 400 μm) to 90.7 ± 2.7% (formulation P6, matrix thickness = 800 μm) and 84.3 ± 3.5% (formulation P7, matrix thickness = 1600 μm). This decrease appears to be due to an increase in the diffusion path length that carvedilol had to travel. Transdermal systems prepared solely with HPMC films (without adhesive coating) exhibited burst release during the first hour of the study and then plateaued. Almost 80% of carvedilol loading was released during the initial 1–2 h (Fig. 1B). Apparently the high hydrophilic character of HPMC matrix (due to its composition i.e., HPMC and propylene glycol) accelerated matrix hydration and swelling leading to the burst effect. This could further be explained by the drug release mechanism suggested by Siepmann and Peppas.

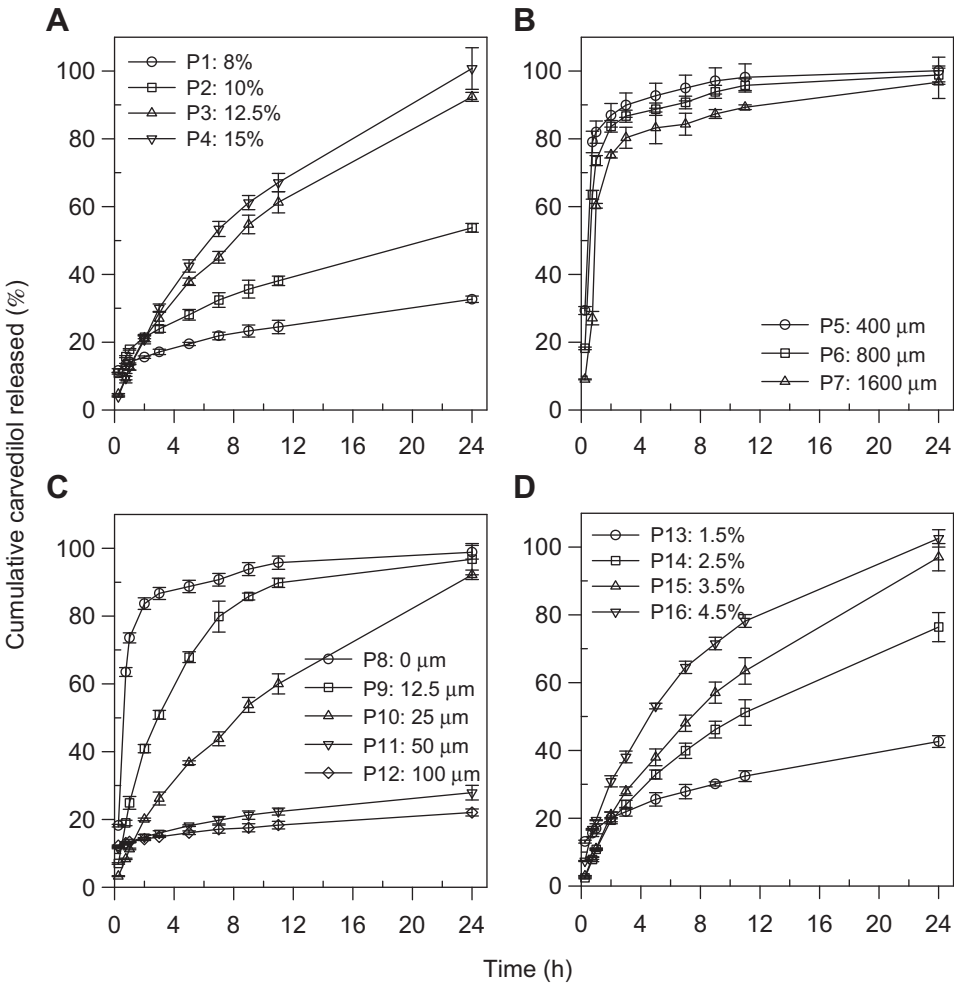

Fig. 1 – Effect of carvedilol loading (A), matrix thickness (B), adhesive layer thickness (C), and propylene glycol concentration (D) on the *in-vitro* release of carvedilol from the patches (Data shown as mean ± standard deviation, $n = 3$).

According to the authors the following steps are involved in the release of drug from the HPMC matrix: (i) water imbibes into the matrix due to initially steep water concentration gradient at polymer/water interface; (ii) this causes the HPMC to swell, resulting in dramatic changes of polymer and drug concentrations, which changes the dimensions of the system; (iii) in the case of high initial drug loadings, the inner structure of the matrix changes significantly during drug release, becoming more porous and less restrictive for diffusion upon drug depletion [10].

The effect of matrix thickness on the permeation profile of carvedilol is shown in Fig. 2B. It was observed that permeation flux value decreased from 115.5 ± 9.3 μg/cm^2/h to 45.1 ± 5.0 μg/cm^2/h with an increase in matrix thickness from 400 μm to 1600 μm. These results are in accordance with the results obtained for the same formulations in the release study.

3.1.3. *Effect of adhesive layer thickness*

A burst release of carvedilol from the patches was observed when the carvedilol-loaded HPMC matrices were fabricated without the incorporation of adhesive layer (formulations P5, P6, and P7). The effect of adhesive layer on carvedilol release from the patch formulations is shown in Fig. 1C. A 12.5 μm adhesive layer could control the burst release thereby decreasing the carvedilol released at 7 h from 90.7 ± 2.7% (formulation P8, without adhesive layer) to 79.8 ± 3.1% (formulation P9, containing 12.5 μm thick adhesive layer). The release was further controlled by increasing the adhesive layer thickness to 25 μm (43.8 ± 2.9% from formulation P10), 50 μm (19.8 ± % 0.9 from formulation P11), and 100 μm (17.1 ± 1.0% from formulation P12). Similarly, carvedilol release decreased progressively from 98.8 ± 3.0% (formulation without adhesive layer) to 22.1 ± 1.7% (formulation containing 100 μm thick adhesive layer). Evaluating carvedilol released at the end of 24 h, it may be concluded that carvedilol release from the patches decreased tremendously with increase in thickness of adhesive layer. This could be attributed to the higher solubility of carvedilol in the adhesive system which reduces the thermodynamic activity of carvedilol in the formulation, which in turn reduces the release of carvedilol [11]. These results are in agreement with a study which attempted to modulate drug release by various formulation variables, the presence and type of adhesive being the main variable [12]. From Fig. 1C, it can be seen that although the initial burst release was efficiently controlled by a 12.5 μm thick adhesive layer, it was not efficient to control the release

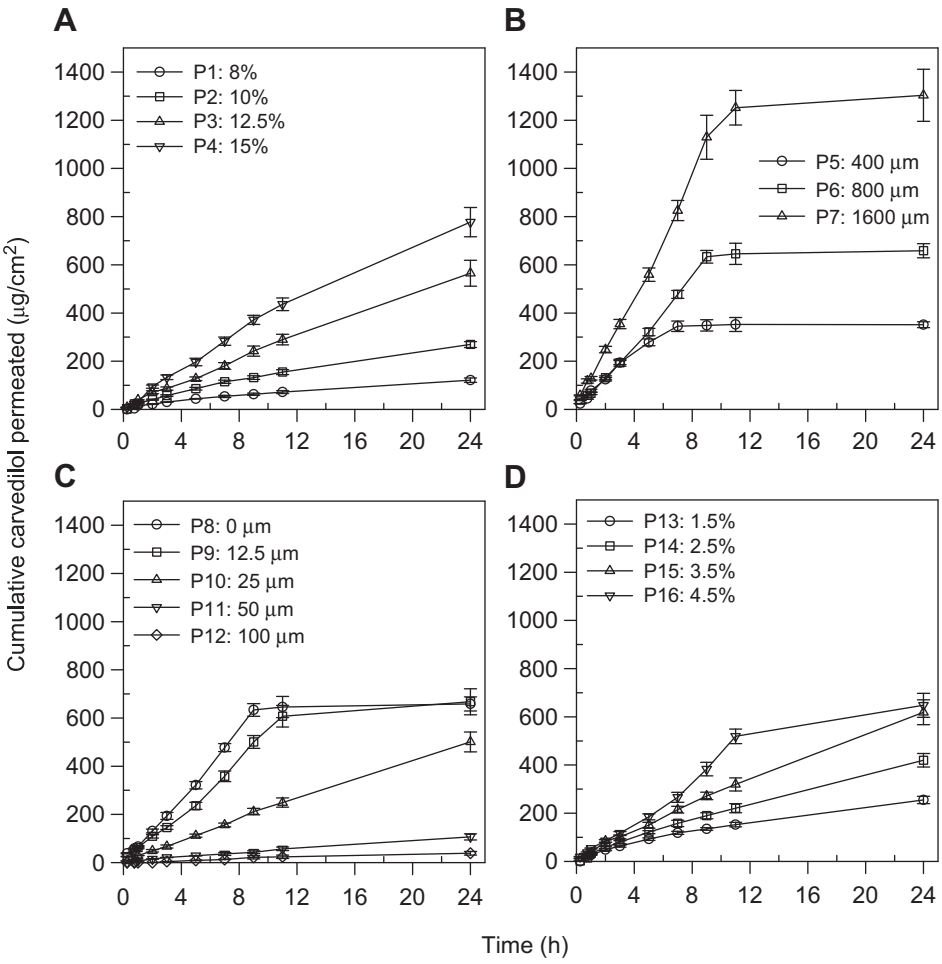

Fig. 2 – Effect of carvedilol loading (A), matrix thickness (B), adhesive layer thickness (C), and propylene glycol concentration (D) on the *in-vitro* permeation of carvedilol across artificial membrane (Data shown as mean ± standard deviation, $n = 3$).

up to 24 h which is required in this study. Thus, a 25 μm thick adhesive layer would be optimum for this study.

Fig. 2C presents permeation profile of carvedilol for various formulations containing different thicknesses of adhesive layer. A significant decrease in permeation flux was observed with an increase in adhesive layer thickness. The permeation flux decreased from 53.9 ± 4.2 μg/cm^2/h from formulation containing 12.5 μm thick adhesive layer (formulation P9) to 1.8 ± 0.1 μg/cm^2/h from formulation containing 100 μm thick adhesive layer (formulation P12). The permeation of carvedilol across the membrane appears to be strongly affected by the thickness of adhesive layer, similar to the pattern observed in the carvedilol release study.

3.1.4. *Effect of propylene glycol concentration*

Propylene glycol was used as a plasticizer to obtain uniform films of HPMC as matrix type of transdermal system. The effect of propylene glycol on the release of carvedilol from the patches is shown in Fig. 1D. Increase in propylene glycol concentration increased the release of carvedilol from the patches. This is evident from carvedilol released at 7 h as well as at 24 h from formulations containing 1.5%, 2.5%, 3.5% or 4.5% propylene glycol. The results in Fig. 1D also indicate that 4.5% of propylene glycol was not efficient in controlling the release of carvedilol up to 24 h. On the other hand, 1.5% and 2.5% of propylene glycol controlled the release of the carvedilol to such an extent that less than 40% of the carvedilol loading was released in 24 h. Thus, 3.5% concentration of propylene glycol would be optimum for this study.

The increase in carvedilol release from the patches with increase in the propylene glycol concentration could be attributed to the high hydrophilic character of propylene glycol which acts as a humectant and leads to more water available in the patches to release carvedilol. A similar study where propylene glycol was used along with HPMC reported that the presence of propylene glycol led to high hydrophilicity of the matrix leading to higher rate of drug release [13]. The reason given for such observation was the formation of hydrophilic micropores in the system aiding water uptake. Other studies have also shown that propylene glycol along with ethanol works as a better release and penetration enhancer [14–19]. Thus, the presence of ethanol used in the preparation of carvedilol solution and propylene glycol in the adhesive layer exhibited synergistic effect.

The permeation of carvedilol across the membrane from various formulations containing different concentrations of

propylene glycol is shown in Fig. 2D. The results indicate that increase in propylene glycol concentration led to an increase in permeation flux from 8.8 ± 3.2 μg/cm^2/h (formulation P13, containing 1.5% propylene glycol) to 41.7 ± 2.8 μg/cm^2/h (formulation P16, containing 4.5% propylene glycol). This is in accordance with the results obtained in the release study. It has been shown that propylene glycol acts as a release as well as a penetration enhancer. Hence, propylene glycol along with ethanol in the adhesive layer further enhanced these effects [18]. The mechanism of permeation enhancing action of propylene glycol is almost similar to that suggested for ethanol. Permeation of the solvent through the membrane could alter thermodynamic activity of the drug in the vehicle which would in turn modify the driving force for diffusion, and the solvent may partition into the membrane facilitating the uptake of the drug in the receptor solution across the membrane [19,20].

In summary, the effect of four formulation variables on carvedilol release during the preliminary study (shown in Fig. 1) suggest the following: (i) The presence of adhesive layer reduced the burst release of carvedilol leading to a better controlled delivery of the carvedilol from the transdermal systems. (ii) Adhesive layer appeared to play a dominating role in controlling the rate of carvedilol release from the patches as evidenced by the tremendous decrease in release rate of carvedilol with a nominal increase in the adhesive layer thickness. (iii) The amount of carvedilol released from the transdermal systems increased with an increase in carvedilol loading and decreased with an increase in matrix thickness. (iv) High carvedilol release could be achieved from the HPMC matrix type of transdermal systems; however, HPMC being very hydrophilic it also leads to burst release of the carvedilol. (v) The propylene glycol used as a plasticizer for HPMC matrix also played a role in controlling carvedilol release from the patches. The release rate of carvedilol from the patches increased as the concentration of propylene glycol increased. (vi) Similar results were obtained when the effect of these four formulation variables was studied on permeation profiles of carvedilol through the artificial membrane, as shown in Fig. 2.

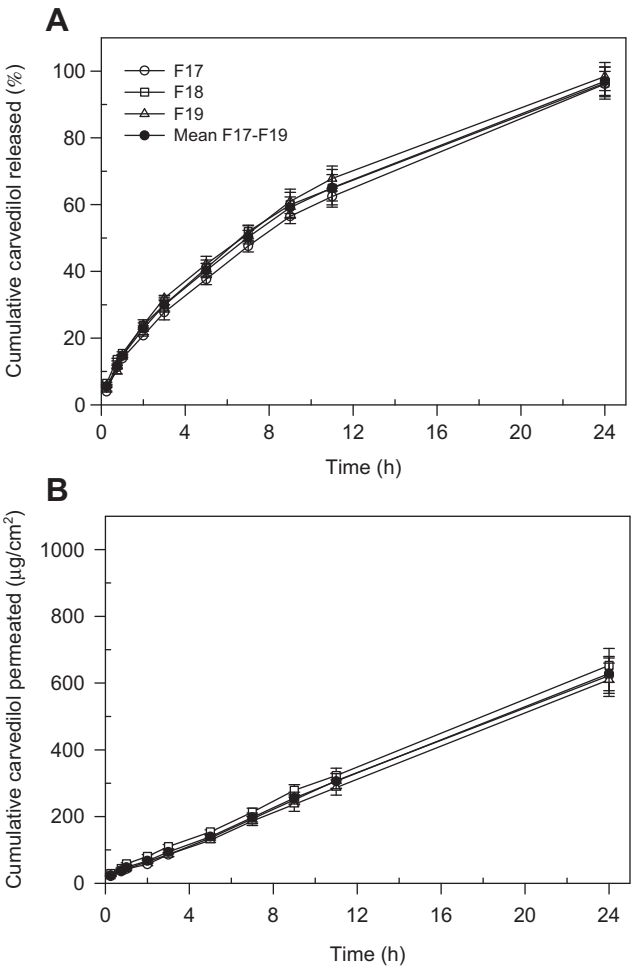

Fig. 3 – Release (A) and permeation (B) profiles of the 3 center points (formulations F17, F18 & F19) and their mean value of the Central Composite IV model (Data shown as mean ± standard deviation, $n = 3$; and Mean F17–F19 $n = 9$).

3.2. *Statistical optimization of the formulation variables*

Based on the conclusions of the preliminary studies, further evaluation of formulation variables was performed using the principle of design of experiments to identify an optimal combination of formulation variables for the fabrication of patches having desired drug release rate and permeation flux. As outlined in Table 3, a total of 19 formulations (formulations F1–P16), conceived from the Central Composite IV model, was studied. The results of drug release as well as permeation profile of carvedilol from carvedilol-loaded transdermal systems are shown in Figs. 3–5. Fig. 3 shows the release and permeation profiles of the 3 central points while Figs. 4 and 5 show the release and permeation profiles of the 8 factorial design points and 8 axial points. Furthermore, the carvedilol released at 7 h and 24 h as well as permeation flux, selected as dependent variables to be used for the regression analysis to identify the optimal formulation of carvedilol-loaded transdermal system, are also summarized in Table 3.

3.3. In-vitro *drug release studies of carvedilol*

Three replicates of the center point (formulations F17, F18 & F19) of the Central Composite IV model were used to evaluate the potential error resulting from experimental conditions instead of formulation variables evaluated. This enables the determination of lack of fit of the suggested regression model. Clustering and overlapping of results from release and permeation profiles of carvedilol shown in Fig. 3 indicate that the experimental error due to the procedure is within the controllable range and the selection of the center point for the experimental design is appropriate.

As shown in Fig. 4, at the end of 24 h, almost 100% of carvedilol loading was released from formulations F1, F3, F5 & F7 (factorial points containing 12.5 μm thick adhesive layer), and formulations F10, F11, F13 & F16 (the axial point formulations). Formulation F13 showed a burst release of carvedilol due to the absence of adhesive layer. Formulation F5 gave the highest carvedilol release rate, which could be attributed to the 12.5 μm thick

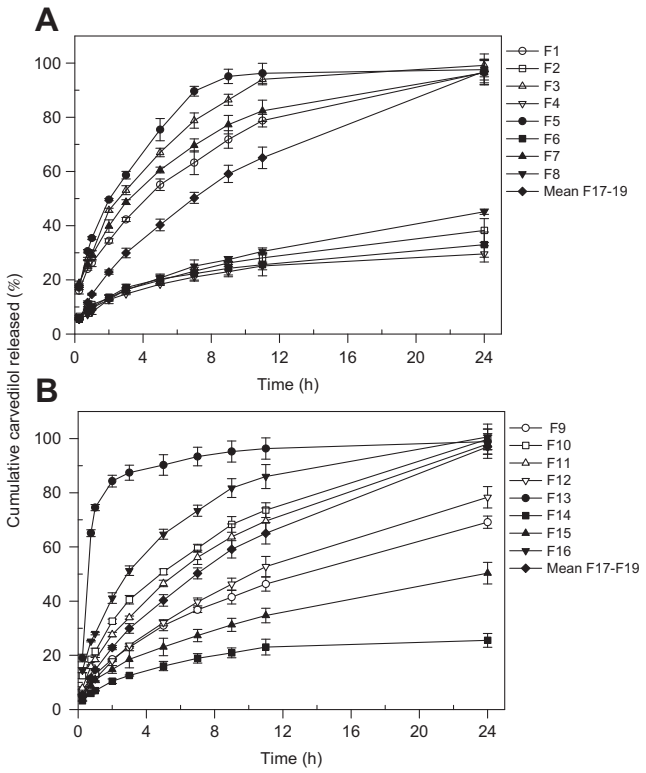

Fig. 4 – Release profiles of 8 factorial points (formulations F1–F8) (A) and 8 axial points (formulations F9–F16) of the Central Composite IV model (Data shown as mean ± standard deviation, $n = 3$; and Mean F17–F19 $n = 9$).

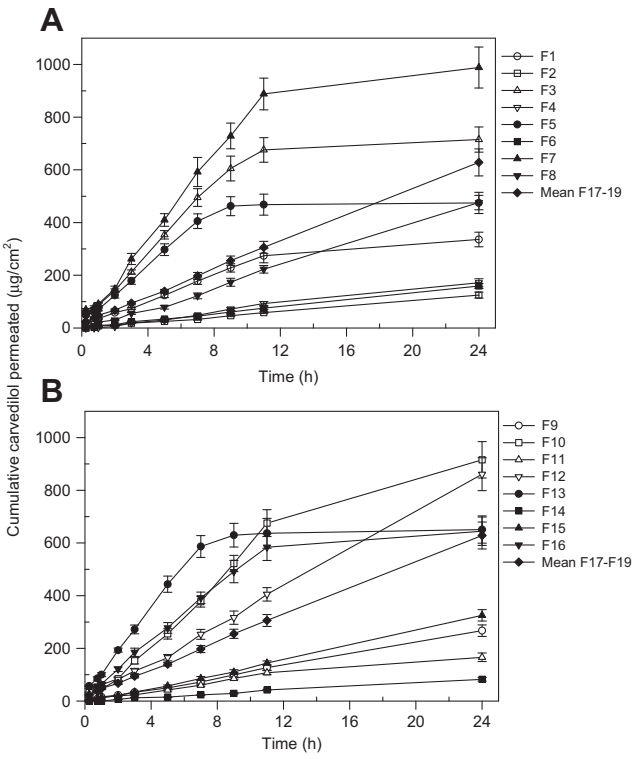

Fig. 5 – Permeation profiles of 8 factorial points (formulations F1–F8) (A) and 8 axial points (formulations F9–F16) of the Central Composite IV model (Data shown as mean ± standard deviation, $n = 3$; and Mean F17–F19 $n = 9$).

adhesive layer, high propylene glycol content, low matrix thickness and high carvedilol loading. In contrast, formulation F14 showed the lowest release rate mainly due to the 50 μm thick adhesive layer. In formulations F8 and F2, though the concentration of propylene glycol was high, release rate of carvedilol was very low mainly due to the thicker adhesive layer. Similar results were obtained for formulation F6 which exhibited very low release rate of carvedilol despite high carvedilol loading. Thus, it may be concluded that the adhesive layer played a dominating role in controlling release of carvedilol from the formulation.

The response parameters obtained from the release profiles of carvedilol from patch formulations to be used for regression analysis are given in Table 3. The values of carvedilol released at 7 h for formulations F2 (23.2 ± 1.9%), F4 (21.0 ± 1.0%), F6 (22.3 ± 1.4%), and F8 (25.1 ± 1.5%) were observed to be lower than 30% of carvedilol loading mainly due to the adhesive layer as discussed in the above paragraph. The values of carvedilol released at 24 h indicate that less than 50% of carvedilol loading was released from the formulations (formulations F2, F4, F6, F8) having high adhesive layer thickness. Furthermore, burst release of carvedilol was seen in formulation F13 (93.4 ± 7.3% released at 7 h) indicating inefficient control of carvedilol release up to 24 h due to the absence of adhesive layer.

3.4. In-vitro *permeation studies of carvedilol*

Permeation profiles of formulations from the Central Composite IV model are shown in Fig. 5. The permeation flux was calculated and the results are summarized in Table 3. Permeation flux values greater than 50 μg/cm²/h were observed for formulations F13 (79.4 ± 5.7 μg/cm²/h), F5 (78.6 ± 5.7 μg/cm²/h), F3 (70.3 ± 6.2 μg/cm²/h), F16 (61.4 ± 2.7 μg/cm²/h), F7 (50.9 ± 4.1 μg/cm²/h), and F1 (50.7 ± 3.2 μg/cm²/h). This might be attributed to the high release rate of carvedilol seen for these formulations and the presence of permeation enhancer/low adhesive layer thickness in these formulations. This confirms the fact that the presence of permeation enhancer and adhesive layer is crucial in permeation of carvedilol to control drug permeation across the membrane.

3.5. *Regression analysis of optimization of formulation*

Based on the values of response parameters summarized in Table 3, backward stepwise regression was performed to generate regression equations for different response parameters. The results of the regression coefficients for each term in the regression model together with the respective correlation coefficient (r^2) of the model are as follows:

For carvedilol released at 7 h (R_{7h}), the quadratic equation can be expressed as Equation (2):

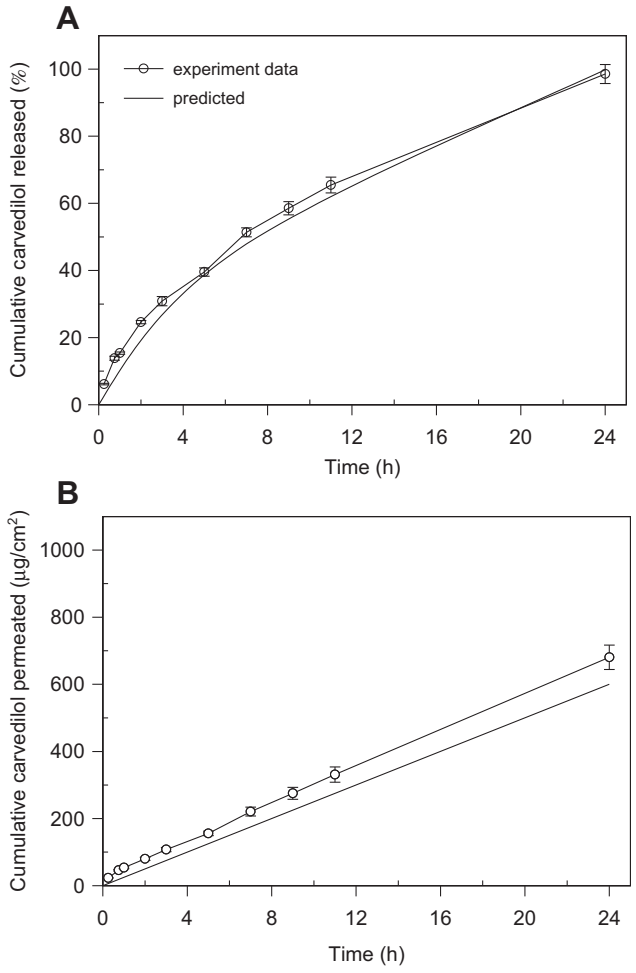

Fig. 6 – Comparison of release (A) and permeation (B) profiles of the optimized formulation and its theoretically predicted profiles (Data shown as mean ± standard deviation, $n = 3$).

$$R_{7h} = 954 + 326X_1 - 190X_2 - 1860X_3 + 709X_4 + 744(X_3)^2 + 188(X_4)^2 - 626X_1X_2 - 318X_1X_3 + 173X_1X_4 \quad (r^2 = 0.9970) \tag{2}$$

For carvedilol released at 24 h (R_{24h}), the quadratic equation can be expressed as Equation (3):

$$R_{24h} = 776 + 260X_1 - 152X_2 - 1449X_3 + 566X_4 + 558(X_3)^2 + 153(X_4)^2 - 501X_1X_2 - 254X_1X_3 + 139X_1X_4 \quad (r^2 = 0.9980) \tag{3}$$

For permeation flux of carvedilol, the quadratic equation can be expressed as Equation (4):

$$\text{Flux} = 4.21 + 0.60X_1 - 2.97X_3 + 0.95X_4 + 0.44(X_3)^2 \quad (r^2 = 0.9183) \tag{4}$$

These three equations indicate the quantitative effect of formulation variables (X_1, X_2, X_3, and X_4) and their interactions on the responses R_{7h}, R_{24h} and permeation flux. The values of the coefficients of X_1, X_2, X_3 and X_4 are associated with the effect of these variables on the response parameters. Coefficients with more than one factor represent an interaction effect, whereas those with higher order terms denote quadratic relationships. A positive sign signifies a synergistic effect, whereas a negative sign stands for an antagonist effect. Only the coefficients that were statistically significant ($P < 0.05$) were retained in the equations. From all the regression equations, it is seen that the regression coefficient of 'X_3' (the adhesive layer thickness) is larger than any other regression coefficient, indicating that thickness of the adhesive layer has dominating role in controlling carvedilol release from the patches as well as permeation of carvedilol across the membrane. This is due to the high lipophilicity of the adhesive layer which reduces diffusivity of carvedilol thereby decreasing the amount of carvedilol released. The values of the coefficients of carvedilol loading and propylene glycol concentration are in accordance with the results obtained in the preliminary results i.e., increase in carvedilol loading and increased propylene glycol concentration increase carvedilol release as well as permeation flux. The regression equation for permeation flux also indicates that matrix thickness does not have a significant effect on carvedilol permeated across the membrane. According to the 3 regression equations, the r^2 value is high indicating the adequacy of the quadratic model.

Since some of the response measurements were competing with each other, a constrained optimization technique was used to generate the optimum setting for the final formulation through proper interplay of different formulation factors. Therefore, the following constraints were used to optimize the formulation: (i) Minimization of the initial/burst release, thus, a 40–45% carvedilol release in the initial 7 h would be favorable. (ii) About 98–100% carvedilol release in 24 h so as to efficiently control the carvedilol release over 24 h. (iii) A permeation flux value of 25 μg/cm²/h. Following the treatment of the constrained optimization, using 47.9% of carvedilol released at 7 h and 99.8% at 24 h as well as 25.7 μg/cm²/h of permeation flux, a formulation having composition of 12.5% carvedilol loading, 1000 μm matrix thickness, 25 μm adhesive layer thickness, and 5%propylene glycol was developed as the optimal formulation of the patch. Excellent correlations were obtained between the observed and predicted values of drug release and permeation (Fig. 6). The results of this regression analysis show that the quadratic mathematical model developed could be used to further predict formulations with desirable release and permeation properties of carvedilol from transdermal systems.

4. Conclusion

The *in-vitro* drug release as well as permeation profiles of carvedilol from transdermal systems were found to be greatly influenced by the formulation variables such as carvedilol loading, matrix thickness, adhesive layer thickness, and propylene glycol concentration and these variables could be suitably altered to achieve the desired controlled release profile of carvedilol. Statistical optimization proved to be very useful in the subsequent formulation development work following preliminary evaluations. The optimization work consisted of three

major parts, narrowing down the formulation variables, generating optimized formulations using Central Composite IV design and optimizing the final formulation using constrained optimization. Thus, the design of experiment with response surface method is an efficient tool to determine and optimize formulation conditions within experimental conditions. Overall, an optimized carvedilol-loaded transdermal system was successfully developed which could control the release as well as permeation of carvedilol up to 24 h.

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